A pre-synopsis entitled as

"Microbial Control of Fungal Pathogens of Brinjal by using Rhizospheric Microorganisms"

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Introduction

Brinjal is the most prominent nutrient-rich tropical vegetables grown in India and it belongs to family *Solanaceae. Solanum melongena* L., is the scientific name of the brinjal, has been grown throughout Asia, Africa, Europe, and the Near East. This fruit is worldwide also known as “eggplant”. In India, brinjal, also known as “baingan” and also known as “aubergine” in North America and Europe, respectively, is a very significant vegetable for everybody. This vegetable displays a broad range of characteristics, including size, shape, and colour, ranging from almost black to yellow, white, green, and purple. This fruit vegetable is good source of vitamins, proteins, and essential minerals (Perrone *et al*., 2012).

Nutritionally, eggplant has low caloric value, high phenolic contents, vitamins, and bioactive compounds minerals like K, Ca, M, P, Na, Fe, and Zn are also beneficial to human health (Sharma & Kaushik, 2021). This crop species has major importance on a global scale. Global eggplant production is concentrated in five leading countries, namely China, India, Egypt, Turkey, and Iran (~93% of the total global production (Alam & Salimullah, 2021).

Brinjal is frequently referred to as "poor man's vegetable" as well as “King of vegetables” due to popularity amongst common people. Regarding area and production, India cultivated brinjal in 760 hectares with production of 12695 metric Tone. (Agricultural Statistics at a Glance 2021, Government of India Ministry of Agriculture & Farmers Welfare Department of Agriculture & Farmers Welfare Directorate of Economics & Statistics).

In India, the brinjal plant is prone to various fungal diseases such as leaf spot (*Alternaria melongenae, A. tenuissima, Cercospora feuilleaboisii, C. solanacea, C. solani, C. solani-melongenae, Colletotrichum dematium C. capsici, C. dematium, Curvularia lunata, Helminthosporium spiciferum, Leptoshaerulina australis, Phyllosticta hortorum, P. macropycnidiai P. solani*), early blight and leaf spot (*Alternaria solani*), leaf and fruit spot (*Ascochyta lycopersici*) on seeds and decaying fruits (*Aspergillus niger, A. parasiticus*), fruit rot (*Chaetomium globosum, Chrysosporium pruinosum, Cladosporium fulvum, Fusarium equiseti, Rhizopus nodosus, R. stolonifer, Trichothecium roseum*), wet rot (*Choanephora cucurbitarum*), black dot of stem and root rot (*Colletotrichum attramentarium, Colletotrichum nigrum*), leaf spot and fruit rot (*Corynespora cassicola, Epicoccum nigrum*), powdery mildew (*Erysiphe cichoracearum, E. polyphaga, Leveillula taurica*), dry rot of stored potato tubers and also wilt (*Fusarium lycopersici*), wilt (*Fusarium solani*), charcoal rot (*Macrophomina phaseolina*), leaf blight and stalk rot (*Myrothecium rordium*), wilt and root rot (*Ozonium texanum*), on seeds and seedling of brinjal (*Pellicularia filamentosa*), white rot, foot and root rot of brinjal (*Pellicularia rolfsii)*, soft rot (*Phomopsis vexans*), on seedlings of brinjal (*Phytophthora colocasiae*), blight of leaf (Phytophthora parasitica), leaf rust, only accidial stage (*Puccinia penniseti*), damping off (*Pythium aphanidermatum*), on seeds (Rhizopus nigricans), rot of stem, root, leaf, petioles, branches and flowering stalks of brinjal (*Sclerotinia sclerotiorum*), warty galls of leaf, petioles and stem (*Synchytrium akshaiberi*), wart disease (*Synchytrium endobioticum*), leaf gall (*Synchytrium melongenae*), wilt (*Verticillium dahliae*) were caused by several pathogens that

reduced the market rate and quality of the crop (Sharma *et al.,* 2013). There are two fungal diseases, Alternaria leaf spot and Fusarium wilt which drastically affect the cultivation and production of brinjal.

There are many practices which are presently being used to control various brinjal pathogens such as physical, chemical, biological, cultural etc. Effective and efficient management of brinjal disease is generally achieved by the use of fungicides but indiscriminate use of fungicides has posed a serious threat to human health and the environment. Due to the increased awareness about the risks involved in the use of fungicides, much attention is being focused on the alternative methods of pathogen control (Babu *et al.,* 2008).

In the negative effects of fungicides, the environmental risks resulting from their indiscriminate use and development of resistance in pathogens have renewed interest towards use of sustainable and ecofriendly approaches for the management of plant diseases like use of biocontrol agents. Rhizospheric microorganisms are the ideal control for Soil borne plant pathogen. Biological control has been developed as an alternative to synthetic fungicide treatment and considerable success has been achieved in utilizing antagonistic microorganisms to control plant diseases (Janisiewicz and Korsten, 2002). Microorganisms as biological control agents have high potential to control plant pathogens and do not have any adverse effect on the environment (Yang *et al.,*2008). Chemical fungicides used to treat fungal disease of plants are toxic to the environment and harmful to human health, so it is necessary to find microbial control by using rhizospheric microorganisms.

Review of Literature

Not much research work has been carried out on the microbial control of Alternaria leaf spot and Fusarium wilt of Brinjal. Still there is need to carry out research work on microbial control of these brinjal diseases.

Fusarium Wilt Disease of Brinjal:

Fusarium oxysporum has the ability to survive in the soil for a longer period of about two decades, and thus it is classified as the most destructive soil-borne fungus that affects most crops, especially eggplant (Gordon and Martyn, 1997). *Fusarium* is the most common soil-borne pathogen that invades many vegetables such as tomatoes, potatoes, peppers, and eggplant. The pathogen *Fusarium oxysporum* attacks plant through the roots and spreads to the stems and leaves, which limits the flow of water, causing the leaves to wilt and turn yellow (Najar *et al.,* 2011). In eggplants, *Fusarium* wilt appears as slight vein clearing on the outer portion of the young leaves often on one side of the plant or on shoot and successive leaves yellow wilt and die, often before the plant reaches maturity. As the disease progresses, plant stunted, and little or no fruit progresses develops. The browning of the vascular system is characteristic of diagnostic of *Fusarium* wilt (Miller *et al.,* 2008; Abdelaziz *et al.,* 2022).

Perusal of earlier research work available on microbial control of *Fusarium* wilt of Brinjal suggested that numerous attempts have been made to exploit fungi and bacteria from rhizospheric soil. Some of these referred herewith:

Earlier Saha, *et al.,* (2012) evaluated 141 antagonistic bacteria isolated from eggplant rhizosphere as biocontrol agents. Out of 141 isolates, two strains AI01 and AI03 which showed maximum antagonistic activity during initial screening against *F. solani* were selected. Both bacterial isolates showed significant reduction of wilt incidence in eggplant seedlings pre-treated with them under greenhouse conditions. Disease incidence was reduced upto 72% when the soil was sterilized before treatment.

In 2010, Akhtar *et al.,* demonstrated that the use of *Bacillus pumilus, Pseudomonas alcaligenes,* and *Rhizobium* sp. improved the growth of *F. oxysporum*-inoculated plants by competing with the pathogen and the production of essential nutrients and antibiotics. Similarly, Yildiz *et al.,* (2012) isolated rhizospheric *Bacillus* and *Pseudomonas* spp. from roots of eggplants collected from different geographical district of Turkey. The isolates designated as 11-4, 13-1, 56K-3, 65A-3, 67A-2, 76A-1, 85A-2, 10a, 318b, 346a, 379c, 007-1, 007-4, and 048-2 were found promising in inhibition of *F. oxysporum* f. sp. *melongenae in vitro* conditions.

Also, Altinok *et al.,* (2013) screened 10 isolates of *Pseudomonas* and *Bacillus* were isolated from eggplant rhizosphere soil and evaluated for their volatile compound efficacy against mycelial growth of *Fusarium oxysporum* f. sp. *melongenae* (Fomg). These PGPR strains inhibited mycelial growth of the target pathogen and the percentage of inhibition varied from 38 % to 72 % by means of inhibitory activities. Among the *Pseudomonas spp. Pseudomonas aeruginosa* (P07-1) and *P. putida* (P11-4) were successful colonizing the eggplant seedlings. Among the PGPR isolates, *P. aeruginosa* (P07-1), *P. putida* (P11-4), *P. aeruginosa* (85A-2), *Bacillus amyloliquefaciens* (76A-1) and *B. cereus* (B10a) significantly reduced Fusarium wilt disease incidence by up to 85 %. However, their combination was not more effective in suppressing the disease than the application of those isolates alone.

In 2018, Abada, *et al.,* tested bioagents and compost tea, caused significant reduction to the linear growth of *Fusarium oxysporum* f. sp. *melongenae* and the germinated conidiospores compared with control treatment. Adding the two tested bioagents, *i.e., B. subtilis* and *P. fluorescens* each alone or in combination to the infested soil with the causal pathogen resulted in significant reduction to eggplant wilt with significant increase to plant height as well as the number of fruits and their weight / plant compared with control treatment. Moreover, amending the soil with compost increased the efficiency of the two bioagents in reducing the disease and increasing the produced fruit yield compared with the clay soil only.

Also, researchers utilized fungal biocontrol agents to control fusarium wilt of brinjal. Some of these followed here, Bashar and Chakma, in 2014 tested Seven soil fungi, such as *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Penicillium sp., Trichoderma harzianum, and T. viride,* for their antagonistic potential against fungi *Fusarium oxysporum and F. solani.* Out of seven fungi, *T. harzianum,* was found to be most effective in inhibiting the growth of both fungi. Five fungicides—agridazim 50 wp, cozeb 80 wp, newban 50 wp, sunvit 50 wp, and vitavax 200 B—were tested for their in vitro effectiveness against *F. solani* and *F. oxysporum* at a concentration of 500 ppm. Of the two test biocontrol fungi , Vitavax 200B was determined to be the most effective inhibitor.

Likewise, Cherkupally, *et al.*, (2017) evaluated the antagonistic activity of seven *Trichoderma* species, against brinjal vascular wilt causing pathogen, *Fusarium oxysporum* f. sp. *melongenae* (Fom) under *in vitro* conditions. The antagonistic activities of seven *Trichoderma* species were screened *in vitro* against Fom by dual culture plate technique and production of volatile and non-volatile metabolites. All the biocontrol agents showed considerable reduction in the growth of the pathogen. Out of the seven fungal antagonists studied for their efficacy, *T. harzianum* showed maximum extent of inhibition 81.11%, followed by *T. koningii* 80.00%, *T. pseudokoningii and T. viride* 78.88% each, *T. virens*, *T. atroviride*, and *T. reesei* 77.77% each by non-volatile compounds. The results of the present study suggest that *T. harzianum* has a highly antagonistic potential against the Fom by production of both volatile and non-volatile compounds. Still there is need to look for potential biocontrol agents against Fusarium wilt pathogen of brinjal.

Alternaria leaf spot disease

Alternaria leaf spot of Brinjal, is caused by *Alternaria melongenae, A. solani* which makes its first appearance in young seedling. It attacks leaves and then spreads to fruits which subsequently rot and become unfit for consumption. Initially disease appears as small, dark brown and sunken spots, which subsequently gets converted into concentric rings and then become olivaceous dark brown lesion due to spore formation. These spots will eventually merge together and completely destroy the leaves. The spots start developing on the lower leaves and work up the plant (Rangaswami & Sambandam, 1960; Bochalya *et al.*, 2012; Raina *et al.*, 2018).

Earlier research work available on microbial control of *Alternaria* Leaf spot of Brinjal is reviewed here.

Some earlier work on microbial control of Alternaria leaf spot of Brinjal is carried out by Ameer *et al.*, in 2021, where they have *in vitro* tested the *Aspergillus niger* and *Purpureocillium lilacinum* against *Alternaria solani*. *A. niger* and *P. lilacinum* at 30% conidia/ml exhibited high inhibitory rate (55.11%) against *A. solani* mycelial growth. Furthermore, these two antagonistic species when applied preventively at 30% of the stock conidial suspension in vivo, significantly reduced disease severity index (<28.41%). Kar *et al.*, (2021) found *Trichoderma harzianum* most effective in reducing the percent disease intensity of brinjal, followed by Neem leaf extract as compared to carbendazim 50 WP (treated check) and control. The maximum yield was found to be in *Trichoderma harzianum*, followed by Neem leaf extract and Datura leaf extract as compared to Carbendazim (treated check) and control.

Also, Reddy *et al.*, (2021) found that the effect of seedling treatment with *T. viride*, neem oil, castor oil, clove oil and its combinations were used to minimize the Alternaria leaf spot disease intensity of brinjal.

All this research work suggests that still needs to be carried out research on microbial control of *Alternaria* leaf spot and *Fusarium* wilt of Brinjal by using antifungal property of microorganism from soil. Hence the present work mainly focused on the rhizospheric

microorganisms soil associated with healthy Brinjal and other plants. The antagonistic activity of indigenous biocontrol agents was evaluated in vitro study.

Objectives:

Brinjal crop has shown higher yield in the last decade but due to the soil deterioration and change in the microbial community in the crop leads to the development of many diseases which cause the losses in yield in upcoming days. The use of chemical fungicides leads to many health problems in animals and humans also, hence it is in need to find out an alternative way to reduce the use of synthetic chemicals for the crop protection and increase the productivity of brinjal. This study mainly focused to find out the antifungal microorganisms against the *Alternaria spp.* causing leaf spot and *Fusarium spp.* causing *Fusarium wilt* of the Brinjal. The chronology of the study is designed given below:

1. To isolate phytopathogen of *Fusarium wilt* and *Alternaria* leaf spot from infected brinjal crop and its pathogenicity testing.
2. To isolate the microorganisms from the rhizospheric soil samples.
3. To screen microorganisms for efficient microbial control agent against isolated phytopathogens.
4. To reveal the mechanism of microbial control agent (in vitro) for their microbial control ability.
5. To formulate microbial control agents.
6. To confirm microbial control ability of selected microbial control agents by (in vivo) pot assay.
7. To study the growth promoting ability of microbial control agents.

Research methodology:

Following are the methods that being used during this research work:

1) **Isolation of *Fusarium* wilt Pathogen**

Fusarium wilt infected brinjal plants were collected from Marathwada region in sterile polythene bags and brought to a research laboratory. Infected stems and branches of Brinjal washed with sterile water thoroughly. The infected stem tissues cut into small pieces (2-5 mm size) and by using flame- sterilized forceps ; they were transferred to sterile petri plates containing 0.1% mercuric chloride solution for 30-60 s. These surface sterilized pieces of stem tissue again transferred to another sterile petri plates containing sterile distilled water to remove rapidly with sterile surgical blade and small pieces from the central core of tissues in the advancing margin of infection cut and aseptically transferred to petri plates containing potato dextrose agar (PDA). Petri plates incubated at room temperature for 6 days (Raut and Hamde , 2016). Isolated pure culture of phytopathogen maintained on PDA and Kings-B agar plate and also on slant for further use.

2) **Isolation of** *Alternaria* **leaf spot pathogen**

Infected leaves of Brinjal plants showing the characteristic symptoms of *Alternaria* leaf spot disease collected in sterile polythene bags from Marathwada region and brought to research laboratory. Leaves cleaned under tap water to remove phylloplane organisms. Each diseased leaf cut into small pieces (5-10mm), washed with sterile distilled water and surface sterilized with 0.1% mercuric chloride for 2 min. Surface sterilized tissues rinsed with three changes of sterile distilled water to remove the traces of $Hgcl_2$. Four pieces transferred on PDA medium with sterile forceps in 90 mm Petri-dishes and then incubated at room temperature for 6-7 days (Raut and Hamde, 2019).

3) **Isolation of Microorganisms from the Rhizospheric Soil Samples:**

Rhizospheric soil samples from healthy plants growing in the fields of Marathwada region were collected in sterile polythene bags and brought to research laboratory. 1 g of each rhizospheric soil sample mixed with 100 ml sterile distilled water and shaken well for 2 min. and then the content of flask allowed to sterile. Different dilutions 10^{-1} to 10^{-6} of these samples prepared by serial dilution technique. Highest dilution 10^{-4}, 10^{-5} and 10^{-6} dilutions used for plating purpose. 0.5 ml selected dilutions plated by using pour plate technique. All the plates incubated at room temperature for 24-48 h (Raut and Hamden, 2016).

4) **Screening of Antifungal activity against phytopathogens:**

The microbial isolates were screened for antifungal activity against phytopathogen by using dual culture technique on king B agar plates (Gull and Hafeez, 2012).

5) **The biocontrol mechanism of rhizospheric isolates**

While finding the mechanism of the microbial control agent, the microbial isolates tested for the production of volatile metabolite, diffusible metabolite siderophore production and lytic enzymes.

6) **Preparation of formulation for the Microbial agents:**

Carrier based powder formulations of microbial control agents will be prepared and used for further study (Rakh, 2010).

7) **Pot Assay for Biocontrol and Growth Promotion**

Using pot assay, the microbial control efficiency and growth promotion efficiency of the selected isolates carried out as per the (Rakh, 2010).

Importance of the study:

The present study mainly focused on finding alternatives to synthetic fungicides which would be ecofriendly, economic, and also health effective to humans as well as animals. The present study mainly focused on the microbial control of phytopathogens of Brinjal by using microorganisms associated with rhizospheric soil.

International Status:

Internationally, several researchers tested and used various microbial agents to control *Alternaria* leaf spot and *Fusarium* wilt of Brinjal. Nuraini *et al.,* (2017) tested Six endophytic

fungi isolated which had antagonist activity against *F. oxysporum*. The antagonistic mechanism of FEB1, FEB2, FEB5 and FED1 was competition; FED2 was antibiosis, and FED3 was parasitism. Based on their morphological characteristics, FEB2, FEB5 and FED3 were identified as *Helicomyces* spp.; FEB1 was a *Rhizopus* sp.; FED1 was a *Mucor* sp.; and FED2 was a species of *Penicillium.*

National status:

Nationally some research work has been undertaken in corresponding to the microbial control of *Alternaria* leaf spot and *Fusarium* wilt of Brinjal. Some of the work has been represented here with.

Najar, *et al.,* (2011) isolated from rhizosphere viz. *Gliocladium roseum* from apple; *Paecilomyces varioti*, *Trichothecium roseum* and *Aspergillus flavus* from mulberry; *Trichoderma viride* (isolate-I&II) of mushroom and pea; *Trichoderma harzianum* (isolate-I &II) of pea and paddy while *Pseudomonas fluorescens* of rajmash and cultured on PDA/king's B medium. All the native BCA's were evaluated in vitro against *Fusarium solani* f. sp. *melongenae* causing wilt disease of brinjal. Except *Gliocladium roseum* and *Trichothecium roseum* all the fungal BCA's were more effective than bacterial BCA i.e.. *Pseudomonas fluorescens,* whereas *Trichoderma harzianum* isolate-I caused maximum inhibition (70.79%) followed by *T. viride* isolate-I(68.57%), *T. harzianum* isolate-II(63.80%), *T. viride* isolate-II(60.0%), *Paecilomyces varioti* (53.33%) and *Aspergillus flavus* (46.34%) but *Trichothecium roseum* and *Gliocladium roseum* were the least effective and resulted 18.09 and 27.61 per cent inhibition of wilt pathogen respectively. The bacterial bioagent, *Pseudomonas fluorescens* was slightly more effective (28.17%) against wilt pathogen. Similarly, *in vitro* agar plug test, *T. viride* (isolate-I&II), *A. flavus, G. roseum* and *T. roseum* were created inhibition zones but rest fungal BCA's were advanced and occupied the entire plate within 10-20 days whereas *P. fluorescens* did not show further advancement beyond the zone of contact.

In 2020, Rao *et al.,* evaluated six bioagents *in vitro* against *F. oxysporum* f. sp *melongenae*, *Trichoderma harzianum* was found effective with maximum inhibition of mycelial growth of the pathogen (67.77 %), followed by *Aspergillus niger* (65.92%). While *B. subtilis* found to be most effective (45.55 %) among antagonistic bacteria under dual culture study. Similar efficacy observed during pot culture study. *Trichoderma harzianum* was found most effective with least pre-emergence seedling mortality (7.66 %) with 84.89 per cent reduction over control. The second and third best antagonists found were *A. niger* and *T. hamatum* which recorded pre-emergence seedling mortality of 11.33 and 13.66 with 77.51 and 64.23 per cent reduction over control respectively.

Similarly, Khursheed *et al.,* (2021) evaluated the efficacy of various bio-agents and plant extracts in vitro. *Trichoderma harzianum* showed highest inhibition among bioagents and among plant extracts Garlic bulb extract showed maximum mycelial growth inhibition. Eco-friendly disease management in the field with most effective bio-agents and plant extracts evaluated in vivo showed that seed treatment with *T. harzianum* and three foliar spray with Garlic clove

extract could effectively manage the disease with lowest disease incidence of 19.13 per cent and intensity of 9.02 per cent respectively and highest fruit yield of 197.80 q/ha, as compared to control plot showing highest disease incidence of 65.51 per cent and intensity of 34.51 per cent respectively with lowest fruit yield of 106.46 q/ha.

Jamil & Kumar (2022) evaluated the effectiveness of combined use of *Trichoderma harzianum* and Carbendazim at a reduced dose against the wilt pathogen on eggplant variety Arka Shirish. *T. harzianum* and Carbendazim are detrimental to wilt pathogen and interfere with its growth. Both *T. harzianum* and Carbendazim were used either as a seed treatment (st) or soil application (sa). Carbendazim (st) produced the highest emergence rate (98.2%); *T. harzianum* (sa)+Carbendazim (st) had the least wilt incidence (16.36%). *T. harzianum* (sa) + Carbendazim (sa) caused the most improvement in plant fresh (169.24 g) and dry (26.35 g) weights and plant length (89.45 cm). Most fruit/plant (19.14), highest yield/plant (858.09 g) and highest phenolic content (98.52 µg catechol·g−1) was due to treatment with *T. harzianum* (sa) +Carbendazim (st); *T. harzianum* (sa) produced the greatest increase in chlorophyll content (3.91 mg·g−1). Integrated use T. harzianum and Carbendazim could reduce wilt incidence in eggplant.

Significance of the study:

The production of Brinjal plant has not been increased due to several biotic and abiotic factors. Chemical fungicides used to treat fungal diseases of plants are toxic to the environment and harmful to human health. The yield of Brinjal Plant is decreased due to many fungal diseases. The major two fungal diseases of Brinjal plants are *Alternaria alternata* and *Fusarium wilt.* This fungal disease is controlled by using Biological control agents like rhizobacteria. Biological control is particularly useful whereas chemical pesticides are not suitable for the environment and also human health. In this study rhizospheric microorganisms play an important role for biological control agents and also plant growth promoting rhizobacteria. *Alternaria* leaf spot and *Fusarium* wilt major disease of Brinjal plant controlled by using microbial control agents i.e. rhizospheric microorganisms.

Work plan:

1) six-month	Completion of Ph. D. Course work
2) six-month	**Isolation of phytopathogens *Alternaria* leaf spot and *Fusarium* wilt from the infected Brinjal crop. Isolation of the Rhizobacteria from the soil. Screening of Rhizospheric isolate for antifungal activity against phytopathogens.**
3) six-month	**Detection of the biocontrol mechanism of Rhizospheric isolates**
4) six-month	**Revealing of the growth promotion abilities of efficient biocontrol isolates**
5) six-month	**Preparation of the formulation of efficient isolates. Pot assay for**

	biocontrol and growth promotion abilities of selected isolates
6) six-month	**Thesis writing and submission**

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